

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:) Prior Application
) Serial No. 08/669,304
) Filed: July 12, 1996 which was a
Peter STOUGAARD et al.) Continuation-in-Part of Serial No.
) 08/476,910, filed June 7, 1995
)
Serial No.: Not yet assigned) Group Art Unit: 1652
)
Filing Date: Not yet assigned) Examiner: William W. Moore

For: RECOMBINANT HEXOSE OXIDASE, A METHOD OF
PRODUCING SAME AND USE OF SUCH ENZYME

Divisional of Serial No. 08/669,304 filed July 12, 1996

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
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Sir:

Prior to examination on the merits of the attached Divisional Application,
please amend the patent application as follows.

IN THE CLAIMS:

Please cancel claims 1-8, 26-31, and 42 without prejudice or disclaimer to the
subject matter therein.

Please amend claims 32, 36, 38-41, and 43-44. Set forth below are the amended
claims in clean form. Also, the amended claims in the marked up form are included
in the attached appendix.

32. (Once Amended) A method of manufacturing a food product wherein
a polypeptide according to claim 9 is used.

36. (Once Amended) A method of manufacturing an animal feed wherein
the polypeptide according to claim 9 is used.

38. (Once Amended) A method of reducing the sugar content of a food product, comprising adding to said product an amount of the polypeptide according to claim 9 which is sufficient to remove at least part of the sugar initially present in said food product.

39. (Once Amended) A method of manufacturing a product selected from the group consisting of a pharmaceutical product, a cosmetic and a tooth care product wherein a polypeptide according to claim 9 is used.

40. (Once Amended) A method of preparing a baked product from a dough, comprising adding the polypeptide according to claim 9.

41. (Once Amended) A dough improving composition comprising a polypeptide according to claim 9, and at least one conventional dough component.

43. (Once Amended) A method of analyzing the content of a sugar in a sample wherein the polypeptide according to claim 9 is used as an analytical reagent.

44. (Once Amended) A method of manufacturing a lactone using a polypeptide according to claim 9, said method comprising applying the polypeptide and/or the microbial cell to a reactor containing a carbohydrate which can be oxidized by the polypeptide and operating the reactor under conditions where the carbohydrate is oxidized to a lactone.

Please add the following claims:

45. (New) A substance having hexose oxidase activity comprising:
a polypeptide characterized by Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) as showing separate bands at 29 kD and 40 kD.

46. (New) The substance of claim 45, wherein the polypeptide is in substantially pure form.

47. (New) The substance of claim 45, wherein the SDS-PAGE comprises a minigel of 12.5% acrylamide/bisacrylamide (37.5:1 mixture) with a thickness of 0.75 mm run in a Mini-Protean II apparatus (Bio-Rad).

48. (New) The substance of claim 47, wherein the SDS-PAGE is run according to Laemmli (1970).

49. (New) The substance of claim 45, wherein the separate bands at 29 kD and 40kD are separate hexose oxidase bands.

50. (New) A substance according to claim 45, wherein the polypeptide comprises at least one amino acid sequence selected from the group consisting of

- (i) Tyr-Glu-Pro-Tyr-Gly-Gly-Val-Pro (SEQ ID NO:1),
- (ii) Ala-Ile-Ile-Asn-Val-Thr-Gly-Leu-Val-Glu-Ser-Gly-Tyr-Asp-X-X-Gly-Tyr-X-Val-Ser-Ser (SEQ ID NO:2),
- (iii) Asp-Leu-Pro-Met-Ser-Pro-Arg-Gly-Val-Ile-Ala-Ser-Asn-Leu-X-Phe (SEQ ID NO:3),
- (iv) Asp-Ser-Glu-Gly-Asn-Asp-Gly-Glu-Leu-Phe-X-Ala-His-Thr (SEQ ID NO:4),
- (v) Tyr-Tyr-Phe-Lys (SEQ ID NO:5),
- (vi) Asp-Pro-Gly-Tyr-Ile-Val-Ile-Asp-Val-Asn-Ala-Gly-Thr-X-Asp (SEQ ID NO:6),
- (vii) Leu-Gln-Tyr-Gln-Thr-Tyr-Trp-Gln-Glu-Glu-Asp (SEQ ID NO:7),

(viii) X-Ile-Arg-Asp-Phe-Tyr-Glu-Glu-Met (SEQ ID NO:8),

where X represents an amino acid selected from the group consisting of Ala, Arg, Asn, Asp, Asx, Cys, Gln, Glu, Glx, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val,

and muteins and variants hereof.

51. (New) A substance according to claim 45, wherein the polypeptide is produced according to a method of producing a polypeptide having hexose oxidase activity, comprising isolating or synthesizing a DNA fragment encoding the polypeptide, introducing said DNA fragment into an appropriate host organism in which the DNA fragment is combined with an appropriate expression signal for the DNA fragment, cultivating the host organism under conditions leading to expressing of the hexose oxidase active polypeptide and recovering the polypeptide from the cultivation medium or from the host organism.

52. (New) A substance according to claim 45, wherein the polypeptide is produced by a microbial cell selected from the group consisting of a bacterial cell, a fungal cell and a yeast cell.

53. (New) A substance according to claim 52, wherein the polypeptide is produced by a cell selected from the group consisting of an *E. coli* cell, a *Saccharomyces cerevisiae* cell and a *Pichia pastoris* cell.

54. (New) A substance according to claim 45, wherein the polypeptide is in a substantially non-glycosylated form.

55. (New) A substance according to claim 45, wherein the polypeptide has functional characteristics identical or partially identical to those of hexose oxidase naturally occurring in *Chondrus crispus*.

56. (New) A substance according to claim 45, wherein the polypeptide shows an enzymatic activity at a pH in the range of 5-9..

57. (New) A substance according to claim 45, wherein the polypeptide has an optimum temperature for enzymatic activity being in the range of 20-60°C.

58. (New) A substance according to claim 45, wherein the polypeptide oxidizes at least one sugar selected from the group consisting of D-glucose, D-galactose, maltose, cellobiose, lactose, D-mannose, D-fucose and D-xylose.

59. (New) A substance according to claim 45, wherein the polypeptide has an isoelectric point in the range of 4-5.

60. (New) A substance according to claim 59, wherein the polypeptide has an isoelectric point of 4.3 ± 0.1 .

61. (New) A substance according to claim 59, wherein the polypeptide has an isoelectric point of 4.5 ± 0.1 .

62. (New) A substance according to claim 45, wherein the polypeptide is in a substantially purified form.

63. (New) A substance according to claim 45, wherein the polypeptide has a molecular weight as determined by gel filtration using Sephadryl S-200 Superfine (Pharmacia) which is in the range of 100-150 kD.

64. (New) A substance according to claim 63, wherein the polypeptide has an apparent molecular weight of $110 \text{ kD} \pm 10 \text{ kD}$.

65. (New) A substance according to claim 45, wherein the polypeptide is part of a fusion product comprising additional enzymatically active amino acid sequences.

66. (New) A method of manufacturing a food product, wherein a polypeptide according to claim 45 is used.

67. (New) A method according to claim 66, wherein the food product is selected from the group of a dairy product, a starch-containing food product and a non-dairy beverage.

68. (New) A method according to claim 66, wherein the polypeptide is acting as an antimicrobial agent or as an antioxidant.

69. (New) A method according to claim 66, wherein the polypeptide is acting as an oxygen removing agent in a food packaging.

70. (New) A method of manufacturing an animal feed wherein the polypeptide according to claim 45 is used.

71. (New) A method according to claim 70, wherein the animal feed is silage.

72. (New) A method of reducing the sugar content of a food product, comprising adding to said product an amount of the polypeptide according to claim 45 which is sufficient to remove at least part of the sugar initially present in said food product.

73. (New) A method of manufacturing a product selected from the group consisting of a pharmaceutical product, a cosmetic and a tooth care product wherein a polypeptide according to claim 45 is used.

74. (New) A method of preparing a baked product from a dough, comprising adding the polypeptide according to claim 45.

75. (New) A dough improving composition comprising a polypeptide according to claim 45 and at least one conventional dough component.

76. (New) A composition according to claim 75, further comprising at least one enzyme selected from the group consisting of a cellulase, a hemicellulase, a xylanase, a pentosanase, an amylase, a lipase and a protease.

77. (New) A method of analyzing the content of a sugar in a sample wherein the polypeptide according to claim 45 is used as an analytical reagent.

78. (New) A method of manufacturing a lactone using a polypeptide according to claim 45, said method comprising applying the polypeptide to a reactor containing a carbohydrate which can be oxidized by the polypeptide and operating the reactor under conditions where the carbohydrate is oxidized to a lactone.

79. (New) A substance having hexose oxidase activity comprising:
a polypeptide characterized by Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) as showing separate bands at 29 kD, 40 kD and 60 kD.

80. (New) The substance of claim 79, wherein the polypeptide is in substantially pure form.

81. (New) The substance of claim 79, wherein the SDS-PAGE comprises a minigel of 12.5% acrylamide/bisacrylamide (37.5:1 mixture) with a thickness of 0.75 mm run in a Mini-Protean II apparatus (Bio-Rad).

82. (New) The substance of claim 79, wherein the SDS-PAGE is run according to Laemmli (1970).

REMARKS

Support for the amendments and added claims is found in the original claims 1-44 and in the specification as a whole, e.g., page 20 and page 38, lines 23-27. As such, no new matter is introduced and entry of the above amendment into the record is respectfully requested.

An indication of allowance of all pending claims is respectfully solicited.

In the event any outstanding issues remain in the application, Applicants would appreciate the courtesy of a telephone call from the Examiner to resolve such issues in an expeditious manner and place the application in condition for allowance.

Respectfully submitted,

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APPENDIX

32. (Once Amended) A method of manufacturing a food product wherein a polypeptide according to claim 9 [or the microbial cell according to claim 29] is used.

36. (Once Amended) A method of manufacturing an animal feed wherein the polypeptide according to claim 9 [or a microbial cell according to claim 29] is used.

38. (Once Amended) A method of reducing the sugar content of a food product, comprising adding to said product an amount of the polypeptide according to claim 9 [or a microbial cell according to claim 29] which is sufficient to remove at least part of the sugar initially present in said food product.

39. (Once Amended) A method of manufacturing a product selected from the group consisting of a pharmaceutical product, a cosmetic and a tooth care product wherein a polypeptide according to claim 9 [or microbial cell according to claim 29] is used.

40. (Once Amended) A method of preparing a baked product from a dough, comprising adding the polypeptide according to claim 9 [or the microbial cell according to claims 29 capable of expressing such a polypeptide to the dough].

41. (Once Amended) A dough improving composition comprising a polypeptide according to claim 9 [or a microbial cell according to claims 29 capable of expressing such a polypeptide in dough], and at least one conventional dough component.

43. (Once Amended) A method of analyzing the content of a sugar in a sample wherein the polypeptide according to claim 9 [or the microbial cell according to claims 29] is used as an analytical reagent.

44. (Once Amended) A method of manufacturing a lactone using a polypeptide according to claim 9 [or a microbial cell according to claims 29], said method comprising applying the polypeptide and/or the microbial cell to a reactor containing a carbohydrate which can be oxidized by the polypeptide and operating the reactor under conditions where the carbohydrate is oxidized to a lactone.

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